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DATA EVALUATION REVIEW

I. Study Type: Aerobic Aquatic Metabolism

II. Citation:

Cohen, Samuel P. 1991. Aerobic Aquatic Metabolism of 2,4-Dichlorophenoxyacetic Acid. Submitted by Industry Task Force on 2,4-D Research Data. Performed by Center for Hazardous Materials Research, Pittsburgh, PA. MRID 42045301.

III. Reviewer:

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Title: Soil Chemist
Organization: Environmental Chemistry Review Section #1
EFGWB/EFED/OPP **18 SEP 1995**

IV. Approved by:

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EFGWB/EFED/OPP **18 SEP 1995**

V. Conclusions:

This study provides supplemental data on the aerobic aquatic metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D). The data are supplemental because low material balances (69 to 70% of applied) were reported for the 27 and 30 day sampling dates. This problem cannot be resolved by submitting additional information. Therefore, a new aerobic aquatic metabolism study is required.

Radiolabeled 2,4-D, at 4.63 $\mu\text{g/g}$, had a first-order degradation half-life of 15 days ($R^2=0.7318$) in a sediment and water system. Similar half-lives of 2,4-D (< 8 days) were reported in aerobic soil metabolism studies (Acc. No. 00116625). Soluble degradates were identified as chlorohydroquinone (CHQ) (0.76 ppm) and 2,4-dichlorophenol (2,4-DP) (0.23 ppm). The major volatile degradate was identified as CO_2 . Radiolabeled residues were also found in nonlabile organic fractions including humic acid, fulvic acid, and humin.

The reported data suggest 2,4-D acid should not persist in aerobic aquatic environments. The dissipation of 2,4-D in aerobic aquatic environments appears to be dependent on oxidative microbial-mediated degradation processes and residue incorporation into bound organic fractions.

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VI. Materials and Methods:

Prior to the aerobic aquatic metabolism experiment, Louisiana rice paddy sediment (clay texture; O.M. = 3.6%; pH=7.3; CEC=28.9 meq 100 g⁻¹; BD=1.20 g/cc) and water were preincubated for 218 days under a humidified atmosphere at a temperature of 24.7°C.

A subsample of preincubated sediment/water (500 gm) was amended with [¹⁴C]-2,4-D (SA=13.0 mCi mmole⁻¹; radiopurity=96.66%) to produce a nominal concentration of 4.63 µg a.i. g⁻¹. The sediment/water mixture was then placed in a 1000 ml incubation flask, which was connected to a series of trapping solutions including ethylene glycol, 1M sulfuric acid, and 5% sodium hydroxide. During incubation, the flask was placed in the dark, was incubated at temperature of 24.7°C, and was continuously purged with CO₂-free air. Sediment and water samples were taken on 0, 2, 5, 12, 20, 27, and 30 days posttreatment. At each sampling period, duplicate samples were taken.

Analytical

Each sediment/water sample was centrifuged to separate the soil and supernatant. Each sediment sample was mixed with H₃PO₄ and then sequentially extracted with anhydrous ethyl ether, D₂O water, and 1N NaOH. The ¹⁴C content in each extract was determined by LSC. Prior to HPLC separations, aliquots of the ethyl ether extract were concentrated to dryness using N₂, and redissolved in water. Duplicate samples of extracted soil samples were further extracted with 0.5N NaOH to separate nonlabile residue fractions.

Soluble residues in sediment extracts were separated using an HPLC equipped with a C18 MICRO PAK column and a linear gradient of 0.1% trifluoroacetic acid (TFA)/water and 1% TFA/acetonitrile; and UV and radioactive detectors set at 280 nm. Soluble residues also were separated using 1-D TLC with a benzene:ethyl acetate:acetic acid (86:10:4 v:v:v) solvent system. Separated residues were identified using co-chromography with 2,4-D, 2,4-DCP, chlorohydroquinone, 1,4 dihydroxy-2-chlorobenzene, 1,2,4-benzenetriol, p-chlorophenoxyacetic acid, and o-chlorophenoxyacetic acid.

The total ¹⁴C content in sediment samples was determined by combustion-LSC. The ¹⁴C content in sediment extracts, water samples, and trapping solutions was determined by LSC. In addition, CO₂ was identified in trapping solutions using BaCO₃ precipitation.

VII. Study Author's Results and/or Conclusions:

A. The material balance of radioactivity ranged from 68.8 to 100.0 % of applied [¹⁴C]-2,4-D. After a 30 day incubation period, the [¹⁴C]-residues were distributed in the water supernatant (14.9% of applied, or 0.69 ppm), sediment (37% of applied, or 1.74 ppm), and trapping solutions (16.4% of

applied, or 0.76 ppm) (Table 1,2,3,4). (Reviewer note: The registrant believes low material balances were caused by inefficient trapping of CO₂.)

B. The estimated first-order half-life of 2,4-D was 15 days ($R^2=0.7318$) in the whole sediment and water system (Figure 9). (Reviewer Note: The calculated half-life does not appear to fit the observed 2,4-D degradation data because only one data point is intercepted by the predicted first-order decay model.)

C. In the aqueous phase, the 2,4-D acid concentrations was 2.61 ppm immediately posttreatment and declined to 0.11 ppm at 30 days posttreatment. In the sediment phase, the 2,4-D acid concentration was 1.92 ppm at 12 days posttreatment and declined to 0.30 ppm at 30 days posttreatment (Table 6).

D. Soluble 2,4-D degradates were identified as CHQ and 2,4-DCP. The major degradate, CHQ, accounted for 16% of applied (0.76 ppm) in the aqueous phase at 27 days posttreatment. The minor degradate, 2,4-DCP, accounted for 4.9% of applied 2,4-D (0.23 ppm) in the sediment phase at 30 days posttreatment. In addition, volatile degradates were trapped in ethyl glycol (0.5% of applied) and 5% NaOH solution gas traps (15.9% of applied). The ¹⁴C-residue in the NaOH trap was identified as CO₂. (Reviewer note: Degradate identification was confirmed using a 1-D TLC separation with a single solvent. One-dimensional TLC with a single solvent system is not accepted as confirmatory identification because residues may co-chromatograph together and hence appear to be a single compound.)

C. At the 27 and 30 day sampling dates, the sediment bound residues were distributed in the ethyl ether extract (11.1 to 13.2% of applied), acid aqueous extract (2 to 3% of applied), acetonitrile/1.5% phosphoric acid extract (0.8 to 3.9% of applied), and nonlabile fractions including humic acid (3.7 to 4.7%), fulvic acid (5.5 to 6.8% of applied), and humin (11% applied).

VI. Reviewer Comments:

A. Low material balances (69 to 70% of applied) were reported for the 27 and 30 day sampling time. The registrant believes low material balances were caused by inefficient trapping of CO₂; however, no analytical explanation for the inefficient CO₂ trapping was given. Similar analytical problems were observed in a previously reviewed anaerobic aquatic metabolism study. EFGWB believes the material balance is too low to provide adequate validation of the laboratory procedures. Therefore, a new aerobic aquatic metabolism study is required.

B. Residue identification was confirmed using a 1-D TLC separation with a single solvent. One-dimensional TLC with a single solvent system is not generally accepted as confirmatory identification because residues may co-chromatograph together and hence appear to be a single compound. In future studies, confirmatory residue identification should be done using at least two different analytical methods.

2,4-D EFED Review

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